2451-Pos Board B437
N-Terminal Truncated Cardiac TnI Extends Frank-Starling Response of the Heart
Hanzhong Feng, Xupei Huang, J.-P. Jin.
Cardiac TnI (cTnI) has an N-terminal extension containing two protein kinase A (PKA) phosphorylation sites, Ser23/24, and its removal by restrictive proteolysis in cardiac adaptation improves myocardial relaxation. Through transgenic expression of the N-terminal truncated cTnI (cTnI-ND) in the heart of endogenous cTnI knockout mice, we studied the function of cardiac muscle containing only cTnI-ND. The pure cTnI-ND hearts showed no hypertrophy or dilation with normal baseline function as compared to wild type controls (WT), confirming the non-destructive nature of cTnI-ND. Echocardiography found increased left ventricular end diastolic dimension in cTnI-ND hearts as compared to WT, implying increased relaxation and compliance in vivo. A series of preloads was tested in ex vivo working hearts for the effects of cTnI-ND on Frank-Starling response. cTnI-ND hearts showed responses to 5-10 mmHg preloads similar to that of WT. When preload was increased from 10 to 20 mmHg, cTnI-ND hearts exhibited better maintained left ventricular relaxation velocity, lower left ventricular end diastolic pressure and larger stroke volume responses than that of WT hearts. 10 nM isoproterenol further increased the positive effects of cTnI-ND on the responses of cardiac function to preloads, indicating that cTnI-ND enhances Frank-Starling relationship in the absence of direct effect of PKA phosphorylation at Ser23/24. Slack sarcomere length in isolated cardiomyocytes and the optimal sarcomere length for maximum tension development in intact left ventricular papillary muscle from cTnI-ND mice were similar to that of WT, suggesting that cTnI-ND extends the range of Frank-Starling response to high preloads by enhancing contractility independent of further increases in sarcomere length. The results demonstrated that the removal of cTnI N-terminal extension by restrictive proteolysis provides a novel posttranslational modification to utilize Frank-Starling mechanism in cardiac adaptation to physiological and pathological stresses.

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Myopathic Splice-Out of the Exon 7 segment of Cardiac Troponin T Predominantly Impairs Systolic Function of the Heart
Hanzhong Feng, Guozhen Chen, Changlong Nan, J.-P. Jin.
The abnormal splice-out of the exon 7-encoded segment in the N-terminal variable region of cardiac troponin T (cTnT-DE7) found in turkeys and dogs with dilated cardiomyopathy significantly reduced the function of transgenic mouse hearts over-expressing cTnT-DE7 (Wei et al., JBC, 2010). Significantly decreased left ventricular peak pressure and contractile and relaxation velocities with elongated isovolumic contraction and relaxation time were seen in ex vivo working hearts from cTnT-DE7 mice in the absence of beta-adrenergic tone, which were correctable by physiological levels of isoproterenol. To further investigate the pathogenic mechanism of cTnT-DE7 under systemic neurohumoral regulation, echocardiography was employed to measure the function of cTnT-DE7 transgenic mouse hearts in vivo. No atrial enlargement, ventricular hypertrophy or dilation was detected in the hearts of 3 months old cTnT-DE7 mice, indicating a compensated state. However, left ventricular fractional shortening and ejection fraction were significantly decreased in cTnT-DE7 mice compared to wild type controls. Under both baseline and beta-adrenergic stimulated conditions, the left ventricular outflow tract velocity and gradient were both significantly decreased in the transgenic mouse hearts, indicating decreased systolic function. The impaired systolic heart function in vivo without changes in diastolic function suggests that cTnT-DE7 primarily reduces contractile function of the cardiac muscle. Interestingly, a restrictive proteolytic deletion of the N-terminal variable region of cTnT (Feng et al., J. Physiol. 2008) was increased in the cTnT-DE7 hearts in vivo. This adaptive response may compensate for the decreased systolic function from the N-terminal abnormality of cTnT-DE7. Our study demonstrated that the N-terminal variable region of cTnT is a regulatory element for cardiac systolic function and a therapeutic target in heart failure.

2453-Pos Board B439
Pathophysiologic Changes Induced by Mutations in the TNT1 Domain of cTnT that Cause FHC
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Familial Hypertrophic Cardiomyopathy (FHC) is a primary cardiac muscle disorder and one of the most common causes of sudden cardiac death in young people. A majority of Cardiac Troponin T (cTnT) mutations are located in the TNT1 domain and cluster at its N- and C-termini. We are investigating the cTnT deletion of glutamine 160 (delta-160E) that is known to be a severe mutation located in a predicted hinge region at the C-terminal end of TNT1. Previous *in vitro* motility studies in our laboratory showed that mutations in this region disrupt weak electrostatic interactions between the thin filament and myosin necessary for strong crossbridge formation. In the current study, we aim to examine the downstream pathophysioloic consequences of this mutation. Cardiac myocytes isolated from hearts of transgenic mice expressing delta-160E cTnT with 35% and 70% replacement and non-transgenic siblings were used to study mechanical function and calcium transients. Our study shows impairments in myocellular mechanics during contraction and relaxation and in the rise and decline of the calcium transient. Furthermore, the alterations in calcium kinetics were dose-dependent. These results support the progressive nature of delta-160E FHC suggested by electron micrographs that demonstrate ultrastructural sarcomeric disarray that increase with transgene expression. In addition, we determined downstream effects of the mutation on expression and function of calcium handling proteins in transgenic mouse hearts using functional assays and immunoblotting. We found that the delta-160E cTnT mutation causes secondary alterations in calcium handling, leading to decreased SR calcium uptake, increased NCX expression, and increased diastolic leak through RyR2. Collectively, these novel findings indicate a phenotype that is distinct from other cTnT mutations and support the need to establish genotype-phenotype links in order to better design molecular therapies to treat FHC.

2454-Pos Board B440
N-Terminal Truncated Cardiac TnI Improves Cardiac Function *In Vivo* and Rescues Restrictive Cardiomyopathy
Pierre-Yves Jean-Charles, Yuejin Li, Changlong Nan, Guozheng Chen, Han-Zhong Feng, J-P Jin, Xupei Huang.
Our previous studies have demonstrated that the phenotype of the transgenic mouse hearts expressing a restrictive cardiomyopathy (RCM) cardiac troponin I (cTnI) C-terminal mutation (R193H) is characterized by a diastolic dysfunction and sudden cardiac death (SCD) (Du et al, 2006, 2008). We have also observed that restrictive cleavage of the N-terminal extension of cTnI (cTnI-ND) that occurs in physiological and pathological adaptations (Yu et al., 2001; Feng et al, 2008; McConnell et al., 2009) desensitizes myofibril sensitivity for Ca^{2+} and enhances diastolic function in transgenic mice expressing cTnI-ND (Li et al, 2010). In the present study, we generated double transgenic mice (Double-TG) expressing different levels of mutant cTnI R193H and cTnI-ND to investigate the dose-dependent rescue effect of cTnI-ND and the mechanisms underlying the protective role of cTnI-ND in young and aged RCM mice. In 2-month-old Double-TG mice, cTnI-ND rescues RCM mice by correcting diastolic dysfunction caused by cTnI R193H mutation in the heart. The rescue effect of cTnI-ND shows a dose-dependent manner. In 8-10-month-old Double-TG mice, echocardiography and Doppler data indicate that cTnI-ND rescues RCM mice not only by reversing the diastolic dysfunction, but also by improving systolic function in the heart, since both diastolic and systolic functions are deteriorated in aged RCM mice. Cell-based assays measuring cardiac cell contractility further confirm the dose-dependent protective effect of cTnI-ND in correcting the impaired relaxation in isolated cardiac myocytes from various Double-TG mouse lines. Consistent with the beneficial effect of cTnI-ND on the function of non-myopathic aging hearts (Biesiadescki et al., 2010), these data demonstrate that cTnI-ND can rescue RCM phenotype not only by correcting diastolic dysfunction in young RCM mice but also by improving systolic function in aged RCM mice.

2455-Pos Board B441
Dose-Dependent Arrhythmia and Cardiac Dysfunction in Restrictive Cardiomyopathy Mice Due to Troponin Mutations
Yuejin Li, Pierre-Yves Jean-Charles, Changlong Nan, Guozhen Chen, Xupei Huang.
Restrictive cardiomyopathy (RCM) is associated with a cardiac troponin I (cTnI) C-terminal mutation (R192H) in human patients. The transgenic mice expressing this mutation have confirmed a phenotype of a diastolic dysfunction and sudden cardiac death (SCD) (Du et al, 2006, 2008). In the present study, we generated transgenic mice ($cTnI^{193His}$) expressing different levels of mutant cTnI R193H (mouse cTnI sequence) to investigate the dose-dependent cardiac dysfunction and to reveal the cause of the death in RCM mice. Our results indicated that the mice ($cTnI^{193His/KO}$ expressing only the mutant cTnI R193H at a wild type cTnI-null background had a dramatic early death at one-month old after birth. Telemetric ECG recording from these mice showed a significant bradycardia starting on day 22 or 23 after birth and a significant ischemia and arrhythmia 1-2 days before death. The diastolic function was deteriorated in these mice determined by echocardiography compared to wild type and the transgenic $cTnI^{193His}$ mice expressing 25% cTnI R193H and 75% wild type cTnI. Cell-based experiments indicated that myocardial contractility decreased significantly corresponding to the content of the mutant cTnI levels in cardiac myocytes and the alteration of Ca^{2+} dynamics in the mutant cTnI cardiac myocytes also showed a dose-dependent manner. Our study has demonstrated that cTnI R193H mutation-caused cardiac dysfunction is dose dependent. Bradycardia is likely an adaptive mechanism of RCM mice to compensate for the prolonged relaxation. The main cause of the death in RCM mice is associated with